Graphene Patterned Microchip For Colorectal Cancer Detection

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ABSTRACT

Cancer currently stands as the second-leading cause of death worldwide. Studies reveal colorectal cancer (CRC) to be the 4th leading cause of mortality due to cancer. It is estimated that about 30% of CRC cases are hereditary, of which 5% are attributed by known syndromes, particularly Lynch Syndrome. Lynch Syndrome (LS) is caused by loss or malfunction of proteins responsible for DNA mismatch repair proteins (MMR), mostly MLH1 and MSH2, causing increased risks of developing CRC. Despite the small percentage accounted with the disease, the severity of the illness still remains immense since 80% of these patients eventually develop CRC and an overwhelming 40-60 % of female patients develop endometrial cancer, the major form of cancer in women in the developing nations. This pilot study aims to fabricate a DNA-graphene-polypyrole (DGP) based biosensor to diagnose deficiency of functional MMR proteins present in patients at a scale of less than ng/ ml. Fundamental understanding of interactions at the interface of biological molecules, such as proteins, and nanomaterials is therefore crucial for developing such biocompatible hybrid materials and biosensing platforms. Conductive nanomaterials-based biosensors offer the advantage of higher sensitivity and reliable diagnosis mainly due to their superior specific surface area and ballistic conductivity. Such films that immobilize proteins can synergize the properties of transducers and molecular recognition elements in order to improve biosensor performance and diversity. Here we report for the first time, the interactions between avidin and a graphene surface, which is being developed as a sensing platform for early detection of DNA mismatch repair proteins. We find that the interactive forces between avidin and graphene are mainly hydrophobic, along with some van der Waals, electrostatic and hydrogen bonding interactions. Notably, the structure and function of the avidin molecule is preserved after its adsorption on the graphene surface. Scanning electron microscopy (SEM) and electrochemical impedance spectroscopy (EIS) analysis of avidin immobilized on a graphenated polypyrrole (G-PPy) conductive substrate, confirms adsorption of avidin on graphene nanoplatelets and corresponding changes in electrical impedance, respectively. EIS analysis of MutS substrate and MutS immobilized on GPPy chips confirmed the working of the bio sensor by corresponding change in electrical impedance.

1. METHOD

1.1 Graphene-Ppy film formation

For this step, we used cyclic voltammetry to co-electropolymerize pyrole and graphene and deposit it on the gold-coated circuit.[1]

CV parameters:

- V_{high}= 900mV
- V_{low} = 800mV
- Scan rate = 20 mV/s
- Number of cycles = 100

1.2 Deposition of Avidin

Avidin molecules were dissolved in a buffer solution of N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid solution (HEPES, 10 mM, pH 7.0). The graphene-Ppy chip was then soaked in the resultant solution for 30 minutes.[2]

1.3 Dilutions of DNA

50µl of 50µM DNA was diluted to form 0.25µM stock solution. For complete saturation of the biosensor with the MutS substrate, the following concentration range is chosen.

Volume taken from the stock solution and diluted to 250µl	Concentration of MutS substrate (µM)
5µl	0.01
50µl	0.1
100µl	0.2
150µl	0.3
200µl	0.4
250µl	0.5

1.4 Dilutions of Protein

100µl of 6.5µM MutS protein was diluted to form 0.5µM stock solution. Dilution is performed in the same way as DNA

Volume taken from the stock solution and diluted to 250µl	Concentration of MutS substrate (µM)
5µl	0.01
50µl	0.1
100µl	0.2
150µl	0.3
200µl	0.4
250µl	0.5

2. RESULTS

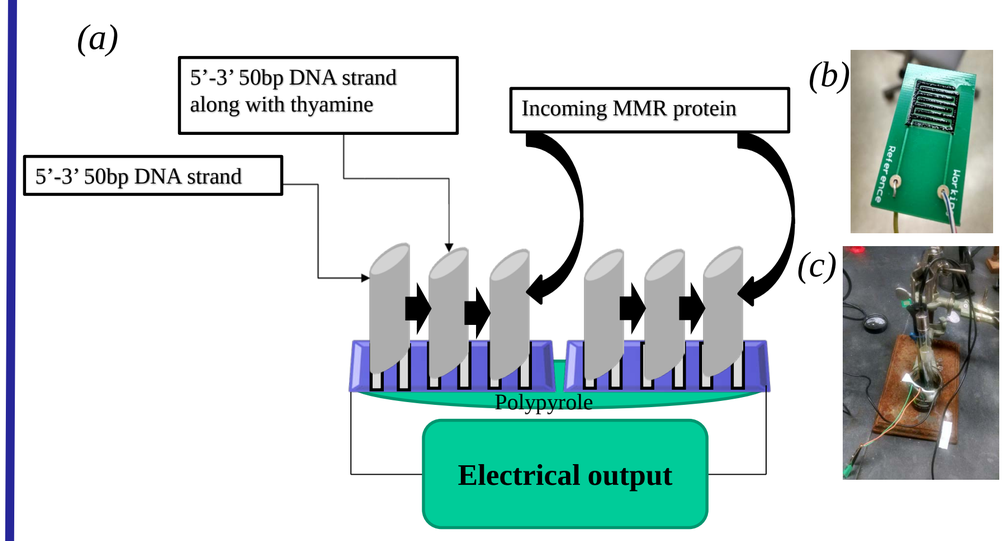

Figure a) Schematic representation of final assembled device; b) Finely deposited grapheme-polypyrole chip c) Electropolymerization of grapheme and pyrole on chip

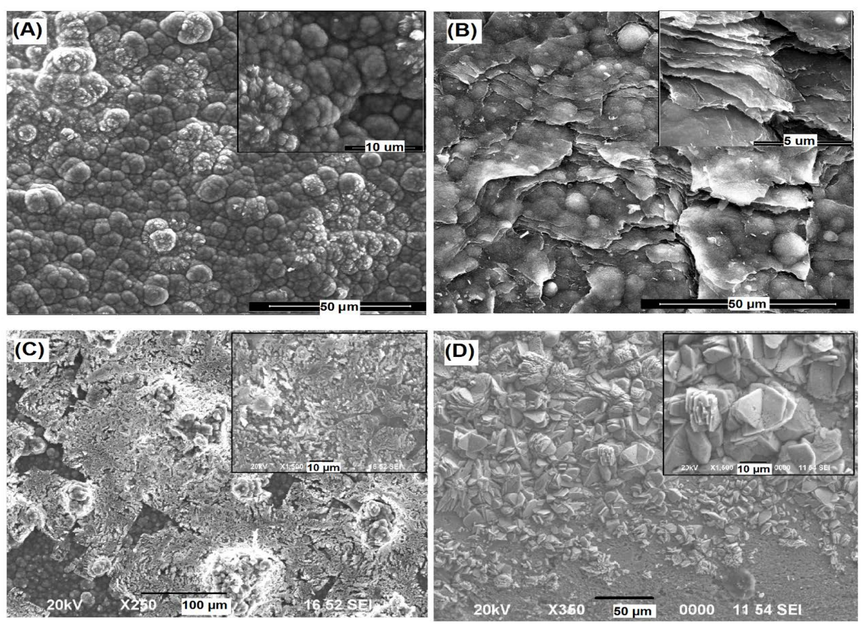

***Figure 2**: SEM analysis of : (A) Control G-PPy surface; (B) High resolution image revealing only embedded graphene flakes; (C) Experimental G-PPy-avidin surface showing greater levels of exfoliated graphene; (D) High resolution image revealing graphene-flakes completely engulfed by avidin molecules.*

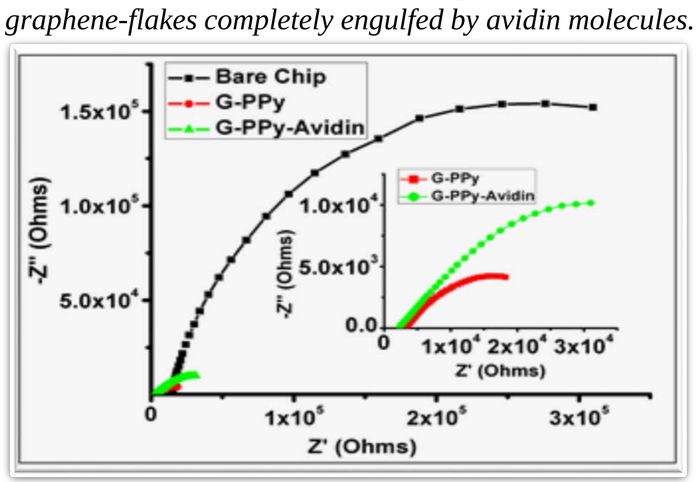

***Figure 3**: EIS analysis of the G/Ppy nanocomposite substrate*

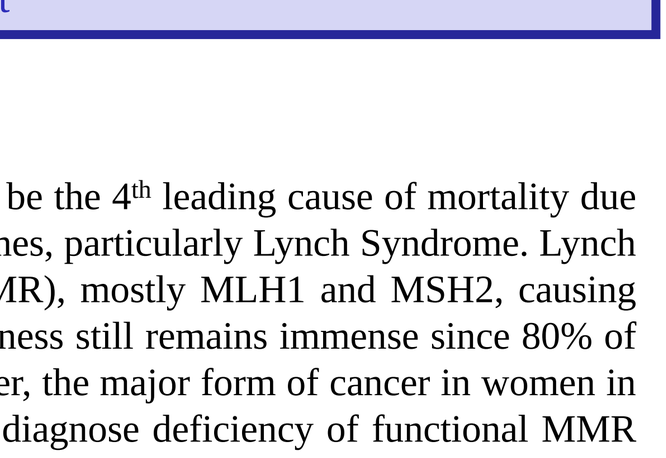

Figure 4: The Nyquist plot demonstrates the change in impedence with different concenttations of MutS

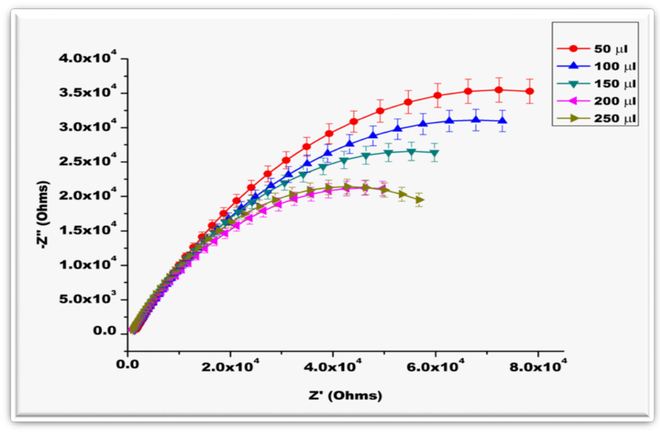

Figure 5: The Nyquist plot demonstrates the change in impedence with different concenttations of MutS Substrate

3. CONCLUSION

We have been successful in depositing avidin onto the graphene-polypyrole substrate, which also shows different conductivity compared to graphene-Ppy only chips. Immobilization of the biotinylated DNA probes has been conducted with optimized concentrations of avidin. Detection of MMR proteins using EIS has also been successful.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

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